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1.	Your reference	9223 GB/JSvn
2.	Patent application number *(The Patent Office will fill in this part)*	0229835.4
3.	Full name, address and postcode of the or of each applicant *(underline all surnames)*	Axis-Shield Diagnostics Limited The Technology Park Dundee DD2 1XA Scotland
	Patents ADP number *(if you know it)*	8531816001
	If the applicant is a corporate body, give the country/state of its incorporation	Scotland
4.	Title of the invention	FACTOR XII VARIANT
5.	Name of your agent *(if you have one)*	Abel & Imray
	"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	20 Red Lion Street London WC1R 4PQ United Kingdom
	Patents ADP number *(if you know it)*	174001

		Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*
6.	If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number	N/A		

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8.	Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:* *a) any applicant named in part 3 is not an inventor, or* *b) there is an inventor who is not named as an applicant, or* *c) any named applicant is a corporate body.* *See note (d))*	to follow

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Signature Abel & Imray Date 20/12/02

Abel & Imray

12. Name and daytime telephone number of person to contact in the United Kingdom Judith Silveston 020 7242 9984

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FACTOR XII VARIANT

INTRODUCTION

The present invention relates to Factor XII, a component of the "contact activation system".

BACKGROUND OF THE INVENTION

Factor XII is an inactive zymogen present in normal blood. It is readily converted, in vitro, in the presence of kallikrein, high molecular weight kininogen and a negatively charged surface into a form, Factor XIIa, that is enzymatically active. The 80Kd form of the serine proteinase, often called Factor αXIIa, has a 52Kd heavy chain linked by a disulphide bond to a 28Kd light chain. Proteolysis of this factor releases a peptide from the heavy chain, and results in a product, Factor βXIIa, that retains serine protease activity, but in which the 28Kd chain of Factor αXIIa is disulphide-linked to a small peptide fragment derived from the former 52-Kd heavy chain. In many cases the small peptide fragment has a molecular weight of about 1000d, but fragments of different size have been observed.

WO90/08835 discloses an immunoassay for Factor XIIa. WO 90/08835 also discloses monoclonal antibodies 2/215 and 201/9, which bind to Factor XIIa, and methods for their production. Monoclonal antibody (mAb) 2/215 is produced by hybridoma 2/215, deposited at the European Collection of Animal Cell Cultures, Divisional of Biologics, PHLS Centre for Applied Microbiology and Research, Porton Down, Salisbury SP4 0JG, England (known as ECACC) on 16 January 1990 under the deposit number 90011606, and

hybridoma 201/9, producing monoclonal antibody 201/9, was deposited at ECACC on 18 January 1990 under deposit number 90012512.

Factor XIIa has long been known to be involved in the contact system of blood coagulation in vivo. More recent work indicates that Factor XIIa is also involved in other systems, including fibrinolysis, kininogensis, and also complement activation and angiogenesis. Many clinical and experimental data are accumulating to suggest that the contact system extends beyond haemocoagulation and that it has a role in maintaining vascular wholeness and blood pressure, that it influences various functions of endothelial cells and that it is involved in control of fibrinolysis and in maintaining the constitutive anticoagulant character of the intravascular space. Further clinical and experimental studies indicate that the contact system is involved in acute and chronic inflammation, shock of different aetiologies, diabetes, allergy, thrombo-haemorrhagic disorders including disseminated intravascular blood coagulation, and oncological diseases. Such conditions, include sepsis, spontaneous abortion and thromboembolism. In addition, Factor XIIa may be involved in tissue defence and repair. Yarovaya et al. (Yarovaya, G.A., Blokhina, T.B. & Neshkova,E.A. Contact system. New concepts on activation mechanisms and bioregulatory functions.Biochemistry (Mosc). 2002 Jan;67(1):13-24) is a recent review of the contact system and new concepts on activation mechanisms and bioregulatory functions.

A number of authors have suggested that activation of Factor XII to XIIa can occur on cell surfaces and have provided data to support that hypothesis. In particular

authors have suggested that activation of Factor XII occurs on cells, notably endothelial cells, through the construction of multi-molecular assemblies that also contain High Molecular Weight Kininogen, Pre-kallikrein and Factor XI. These models indicate that, after it has been activated, Factor XIIa dissociates from the assembly and does not remain on the cell surface for a prolonged time, see for example, Yarovaya et al. (loc. cit.).

SUMMARY OF THE INVENTION

The present invention is based on our surprising observation that activated Factor XII (Factor XIIa) is present on the surface of cells circulating in the blood. This observation is contrary to the previous findings described above that, after activation in a multi-molecular assembly on a cell surface, Factor XIIa dissociates from the assembly and does not remain bound to the cell.

A further observation is that, when Factor XIIa is cellular, not all Factor XIIa epitopes appear to be as accessible as when Factor XIIa is not cellular. For example, monoclonal antibody 2/215 is capable of binding effectively to cellular Factor XIIa and to non-cellular Factor XIIa. However, monoclonal antibody 201/9 and a sheep polyclonal antibody raised against Factor βXIIa do not appear to be able to bind as effectively to cellular Factor XIIa as to non-cellular Factor XIIa.

The term "cellular Factor XIIa" and "cellular Factor XII" are used herein to denote Factor XIIa and Factor XII, respectively, present on the surface of a cell.

The present invention provides a method which comprises detecting or determining Factor XII or a fragment thereof, for example, Factor XIIa, in a sample comprising cells obtained from a mammalian subject, particularly cells circulating in blood or other body fluids.

The present invention provides a monoclonal antibody that is capable of binding to cellular Factor XII or a fragment thereof, for example, cellular Factor XIIa, for example, a monoclonal antibody having the same or similar properties as mAb 2/215 in relation to binding to cellular Factor XII, especially to cellular Factor XIIa, but not including mAb 2/215 itself.

The present invention also provides a method for producing a monoclonal antibody that binds to cellular Factor XII or a fragment thereof, which comprises raising monoclonal antibodies against Factor XII or a fragment thereof and screening the antibodies against cellular Factor XII or fragment thereof.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the protocol used to carry out an immunoassay for cellular Factor XIIa using the IMx system of Abbott Laboratories.

Figure 2 shows the normalised response for three different sample types, namely cell rich plasma, cell poor plasma and washed cells, on a microtitre plate Factor XIIa assay, using mAb 2/215 as capture antibody and labeled polyclonal antibody (polyclonal conjugate) and labeled mAb 2/215 (2/215 conjugate) for detection of cellular Factor XIIa.

Figure 3 shows the response for three different sample types, namely cell rich plasma, cell poor plasma and cell suspension, on a microtitre plate Factor XIIa assay, using mAb 2/215 as capture antibody and labeled polyclonal antibody (polyclonal conjugate) and labeled mAb 2/215 (2/215 conjugate) for detection of cellular Factor XIIa.

Figure 4 shows the response for three different sample types, cell rich plasma, cell poor plasma and cell suspension, in an IMx Factor XIIa assay, using mAb 2/215 as capture antibody and labeled mAB 201/9 (201/9 conjugate) and labeled mAb 2/215 (2/215 conjugate) for detection of cellular Factor XIIa.

Figure 5 shows flow cytometry data obtained for FITC labeled mAb 2/215 incubated with plasma. Figure 5a shows data obtained for the plasma in the absence of labeled antibody, Figure 5b shows data obtained when plasma was incubated with the labeled antibody. The shift in the distribution indicates that the labeled 2/215 antibody binds to a cellular component of plasma.

Figure 6 shows the cellular Factor XIIa content of plasma for 8 individuals as determined by the addition of radiolabelled mAb 2/215.

Figure 7 shows the response for three different sample types, namely cell rich plasma, cell poor plasma and cell suspension, from an individual totally deficient in Factor XII. The Factor XIIa assay was performed on an IMx analyser, using mAb 2/215 as capture antibody and 201/9 and 2/215 conjugates.

Figure 8 shows the normalised response for three different sample types, namely cell rich plasma, cell poor plasma and cell suspension, from an individual totally deficient in Factor XII. The Factor XIIa assay was performed on an IMx analyser, using mAb 2/215 as capture antibody and 201/9 and 2/215 conjugates.

Figure 9 shows the normalised response for three different sample types, namely cell rich plasma, cell poor plasma and cell suspension, from an normal volunteer and from an individual totally deficient in Factor XII. The Factor XIIa assay was performed on an IMx analyser, using mAb 2/215 as capture antibody and 201/9 and 2/215 conjugates.

Figure 10 shows the normalised response for three different sample types, namely cell rich plasma, cell poor plasma and cell suspension, from an normal volunteer and from an individual totally deficient in Factor XII. The Factor XIIa assay was performed on an IMx analyser, using mAb 2/215 as capture antibody and 201/9 and 2/215 conjugates.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a method which comprises detecting or determining Factor XII or a fragment thereof, for example, Factor XIIa, in a sample comprising cells obtained from a mammalian subject, generally a human, particularly cells circulating in blood or other body fluids.

Depending on the assay system used, the sample may comprise of a body fluid containing cellular material in addition to the aqueous phase, or the sample may comprise

isolated cells, that is to say, cells substantially free from the liquid phase in which they exist in vivo, or the sample may comprise tissue or cells obtained from a tissue sample.

Methods for detecting or determining non-cellular Factor XII or a fragment thereof, for example, non-cellular Factor XIIa are known and include chromogenic, for example, amidolytic assays and various types of immunoassays, for example, as described in more detail below. Any such assay may be used, provided it is also capable of detecting or determining cellular Factor XII or a fragment thereof, for example, cellular Factor XIIa.

An assay known to suitable for detecting or determining non-cellular Factor XII or a fragment thereof, for example, non-cellular Factor XIIa may be tested for the ability to detect or determine cellular Factor XII or a fragment thereof, for example, cellular Factor XIIa in a sample.

For example, using a sample known to comprise cellular Factor XII or a fragment thereof, for example, cellular Factor XIIa, the results obtained for an assay under investigation are compared with the results obtained using an assay known to be suitable for the detection of cellular Factor XII or a fragment thereof, for example, cellular Factor XIIa. Monoclonal antibody 2/215 is capable of binding effectively to cellular Factor XIIa. An immunoassay involving mAb 2/215 may be used as a comparison assay.

An alternative is to carry out the assay under investigation on a portion of a sample known to comprise

cellular Factor XII or a fragment thereof, for example, cellular Factor XIIa. The sample should not contain non-cellular Factor XII or a fragment thereof, for example, non-cellular Factor XIIa. Another portion of the sample is treated to release the Factor XII or the fragment thereof, for example, Factor XIIa from the cells, the treated cells are isolated, the assay is repeated, and the results of the two assays are compared. If result obtained for the assay on the sample that contains cellular Factor XII or a fragment thereof, for example, cellular Factor XIIa is higher than that obtained from the sample treated to remove the cellular Factor XII or fragment thereof, for example, cellular Factor XIIa, that indicates that the assay is suitable for detecting or determining cellular Factor XII or a fragment thereof, for example, cellular Factor XIIa.

The term "antibody" as used herein includes any antibody fragment that is capable of binding antigen, for example, Fab and $F(ab')_2$ fragments, and also recombinant, chimeric and humanized antibodies.

Measurement of cellular Factor XII or a fragment thereof, for example, cellular Factor XIIa may be performed on a sample of a body fluid, for example, whole blood or plasma. Alternatively, cells may be isolated from a sample of a body fluid, for example, whole blood or plasma prior to analysis to determine the cellular Factor XII or a fragment thereof, for example, cellular Factor XIIa. Cells may also be obtained from a tissue sample.

Cells may be isolated, for example, by centrifugation and washing. Preferably the cells are centrifuged and washed at least one, preferably two or more times.

Centrifugation should generally be carried out under sufficiently high g forces that the cells form a discrete pellet that can be separated from the supernatant. The pellet may be washed in a suitable medium that does not affect the cellular Factor XIIa, for example, that does not cause the cellular Factor XIIa to become dissociated from the cell. For example, phosphate buffered saline pH7.4 may be used for washing and for suspension of cells for the detection or determination of cellular Factor XIIa.

If the assay used is capable of detecting or determining both cellular and non-cellular Factor XII or a fragment thereof, for example, Factor XIIa, carrying our the assay on a sample comprising cells will detect or determine will give a result for both the cell-bond and the non-cellular analyte. However, if the assay is carried out on a sample of isolated cells, the result will be for cellular analyte only. The term "a sample comprising cells" is used herein to denote include samples of body fluids that comprise cells and samples of isolated cells.

An immunoassay may be used to detect or determine cellular Factor XII or a fragment thereof, for example, cellular Factor XIIa in a sample comprising cells, for example, by contacting the cells with an antibody, especially a monoclonal antibody, that is capable of binding to cellular Factor XII or a fragment thereof, for example, cellular Factor XIIa, and detecting or determining any resulting antigen-antibody complex.

Antibodies capable of binding to Factor XII may be produced in a conventional manner using Factor XII as antigen and screening against Factor XII. Anti-Factor

XII antibodies are known, and an immunoassay for Factor XII is available commercially from Enzyme Research Laboratories (15 Sketty Road, Swansea, UK). Anti-Factor XIIa antibodies are also known, see above. mAb 2/215 or another monoclonal antibody having the same or similar Factor XIIa binding properties as mAb 2/215 may be used.

Methods of carrying out immunoassays are well known, see for example, Methods in Enzymology, H. Van Vunakis and J. J. Langone (Eds), 1981, 72(B); Practice and Theory of Enzyme Immunoassays, P Tijssen, Laboratory Techniques in Biochemistry and Molecular Biology, R. J. Burden and P. H. Van Knippenberg (Eds), Elsevier, 1985; Introduction to Radioimmunoassay and Related Techniques, T. Chard, ibid, 3rd Edition, 1987; and Methods in Enzymology, H. Van Vunakis and J. J. Langone (Eds) 1981, 74(C).

Immunoassay techniques, both qualitative and quantitative, include ELISA (enzyme linked immunosorbent assays), Western blotting, fluid phase precipitation assays, coated particle assays, competitive assays, sandwich assays, including forward, reverse and simultaneous sandwich assays, and solid phase radio immunoassays (SPRIA).

In one ELISA format that may be used according to the present invention, a capture antibody, especially a monoclonal antibody, that is capable of binding to cellular Factor XII or a fragment thereof, for example, cellular Factor XIIa, is immobilized on a solid phase support, for example, on a plastics or other polymeric material, for example on the wells of plastics microtitre plates, or on beads or particles, for example, as used in proprietary systems, for example, the IMx system of

Abbott Laboratories, Abbott Park, Illinois USA. Samples comprising cells are incubated in contact with the immobilised capture antibody and any resulting captured cellular Factor XII or a fragment thereof, for example, cellular Factor XIIa is detected using a labeled antibody that is capable of binding to the cellular XII or fragment thereof, for example, cellular Factor XIIa.

The labelled antibody may be polyclonal or monoclonal. Anti-human antibodies, for example, anti-human polyclonal antibodies, are often convenient for use as labelled antibodies. The label may be detectable directly or indirectly. Any appropriate radioisotope may be used as a directly detectable label, for example a β-emitter or an γ-emitter, examples being ^{125}I, ^{131}I, ^{3}H, and ^{14}C. For commercial use, non-radioactive labels, generally enzyme labels, are preferred. Enzyme labels are detectable indirectly. An enzyme label is, for example, alkaline phosphatase or a peroxidase, for example, horse radish peroxidase. An appropriate substrate for the chosen enzyme, for example, a substrate that gives rise to a detectable optical or fluorescence change, for example, phenolphthalein monophosphate or a fluorescent substrate, for example, methyl umbeliferone, is used. Alternatively, there may be used an enzyme reaction that can be followed using an electrochemical method.

Factor XII or an antigenic fragment thereof, for example, Factor XIIa, that is labeled, for example, radiolabelled or enzyme-labelled, may be used in a competitive assay for cellular Factor XII or a fragment thereof, for example, cellular Factor XIIa. The term "an antigenic fragment of α-XIIa or Factor βXIIa, and other, for example, smaller, fragments of Factor XII that retain the

ability to bind to an appropriate antibody, for example, a anti-Factor XII or anti-Factor XIIa antibody.

An example of an ELISA for Factor XII is an immunoassay that utilizes an anti-Factor XII antibody immobilized on a solid support to capture Factor XII and detecting or determining the captured Factor XII. Such an assay may be carried out using materials provided by Enzyme Research Laboratories (15 Sketty Road, Swansea, UK). This assay is based upon capturing Factor XII using a solid-phase affinity purified goat anti-Factor XII IgG antibody. The amount of Factor XII bound to the solid phase can be demonstrated using a goat anti-Factor XII antibody conjugated to peroxidase.

An example of an immunoassay for Factor XIIa is that described in WO90/08835. To determine cellular Factor XIIa it is recommended that mAb 2/215 is used, especially as the capture antigen. A different antibody, for example, a polyclonal antibody or a different monoclonal antibody may be used for detection.

Further methods utilise direct detection of a resulting antibody-antigen complex. Examples of such techniques are Surface Plasmon Resonance, Surface Acoustic Wave and Quartz Crystal Microbalance methodologies (Suzuki M, Ozawa F, Sugimoto W, Aso S. Anal Bioanal Chem 372:301-4, 2002; Pearson JE, Kane JW, Petraki-Kallioti I, Gill A, Vadgama P. J Immunol Methods ;221:87-94, 1998; Weisch W, Klein C, von Schickfus M, Hunklinger S. Anal Chem 1996 68:2000-4, 1996; Chou SF, Hsu WL, Hwang JM, Chen CY. Clin Chem 48:913-8, 2002).

The materials used act as standards and controls may take various forms dependent upon the assay to be used. In some assay formats, suitable material may be in the form of aqueous Factor XII or a fragment thereof, including the various forms of activated Factor XII. In other formats, for example where the same antibody is used as the capture and detection (conjugate) antibody in an ELISA, it may be desirable to create constructs containing multiple Factor XII molecules or fragments thereof, including the various forms of activated Factor XII, for example, by binding Factor βXIIa to the surface of beads, for example, polycarbonate beads, for example, 3 μM in diameter.

Cellular Factor XII or a fragment thereof, for example, **cellular** Factor XIIa may be detected or determined by flow cytometry using an appropriate antibody having a suitable label, for example, fluorescein isothiocyante (FITC). Appropriate anti-Factor XII antibodies are described above, as are anti-Factor XIIa antibodies, for example, mAb 2/215 or another monoclonal antibody having the same or similar properties as mAb 2/215 in relation to binding to cellular Factor XIIa.

Alternatively, Factor XII or a fragment thereof, for example, Factor XIIa in a sample comprising cells may be determined by means of other types of assay, including clotting assays and amidolytic assays, for example, chromogenic assays.

Factor XIIa may be determined by measuring its enzyme activity using a chromogenic substrate, for example, as described by Vinazzer H., Thromb Res., 14, 155-66, 1979.

The invention, especially the immunoassays described above, provides a rapid method of determination of cellular Factor XII or fragments thereof, for example Factor XIIa, that can be used readily on automated equipment for large scale use.

The presence of cellular Factor XII or a fragment thereof, for example, cellular Factor XIIa, in a tissue sample may be detected using an immunohistological technique. For example, a monoclonal antibody as described above, labeled with an appropriate label, for example, a fluorescent label, may be used.

Monoclonal antibodies and immunoassays according to the present invention may be used in studies of coagulation systems and of thrombotic and other disorders, see also below.

An immunoassay was carried out using mAb 2/215 as both capture and detection antibody, with cell rich plasma, cell poor plasma and washed cells obtained from plasma as samples. The cell rich plasma was obtained by centrifuging citrated blood from 10 minutes at 1000g. High speed centrifugation of the cell rich plasma at 16,000g for 10 minutes gave a supernatant, called cell poor plasma. The precipitate was washed in 100 mM phosphate buffered saline pH 7.4 (PBS) and centrifuged at high speed (16,000g for 10 minutes) three times and then suspended in PBS to give "washed cells". Using mAb 2.215 as both capture and detection antibody, the maximal response was obtained for the washed cells, with a minimal response for the cell poor plasma. In contrast, when a polyclonal antibody was used as the detection antibody with mAB 2/215 as the capture antibody,

significant responses were obtained for both the cell rich and cell poor plasmas, but only a minimal response for the washed cells. Further immunoassay and flow cytometry experiments confirmed the results. These results indicate that mAb 2/215 binds to epitope(s) on Factor XIIa that are available when the Factor XIIa is cellular, whereas the epitope(s) on Factor XIIa to which mAb 201/9 bind are less available for binding when the Factor XIIa is present on the surface of the cell.

The present invention further provides a kit for carrying out an immunoassay of the present invention, which kit comprises, each in a separate container or otherwise compartmentalised: (i) a monoclonal antibody that is capable of binding to cellular Factor XII or a fragment thereof, for example, cellular Factor XIIa, for example, mAb 2/215 or another monoclonal antibody having the same or similar Factor XIIa binding properties as mAb 2/215, and (ii) a labeled antibody capable of binding to cellular Factor XII or a fragment thereof, for example, cellular Factor XIIa, when the Factor XII or a fragment thereof is bound to the monoclonal antibody defined in (i).

The kit may comprise further components for carrying out an immunoassay, for example, as described above. The monoclonal antibody may be immobilised on a solid support.

A kit according to the invention may comprise, for example,

a) a monoclonal antibody that is capable of binding to cellular Factor or a fragment thereof, for example, cellular Factor XIIa, for example, mAb 2/215 or another

monoclonal antibody having the same or similar Factor XIIa binding properties as mAb 2/215,

(b) a suitable material to act as standard, and

(c) labelled antibody capable of reacting directly or indirectly with cellular Factor XII or a fragment thereof, for example, Factor XIIa, when the Factor XII or a fragment thereof is bound to the monoclonal antibody defined in (i).

The materials used act as standards and controls may take various forms dependent upon the assay to be used. In some assay formats, suitable material may be in the form of aqueous Factor XII or a fragment thereof, including the various forms of activated Factor XII. In other formats, for example where the same antibody is used as the capture and detection (conjugate) antibody in an ELISA, it may be desirable to create constructs containing multiple Factor XII molecules or fragments thereof, including the various forms of activated Factor XII, for example, by binding Factor βXIIa to the surface of beads, for example, polycarbonate beads, for example, 3 μM in diameter.

Alternatively, a kit may comprise labeled Factor XII or a fragment thereof, for example, Factor XIIa, for use in a competitive assay.

A kit may also comprise further components, each in a separate container, for example, diluent(s), wash reagent solution(s)and substrate solution(s).

The present invention also provides an assay device suitable for carrying out an assay of the invention. The term "assay device" is used herein to denote means for

carrying out an immunoassay comprising a solid phase, generally a laminar solid phase, for example, a membrane, sheet, strip, coating, film or other laminar means, on which is immobilized an appropriate capture antibody, for example, a monoclonal antibody as described above. The immobilized antibody is preferably present in a defined zone, called herein the "antigen capture zone".

An assay device may incorporate the solid phase within a rigid support or a housing, which may also comprise some or all of the reagents required for carrying out an assay. Sample is generally applied to an assay device at a predetermined sample application zone, for example, by pouring or dripping the sample on the zone, or by dipping the relevant part of the device into the sample. If the sample application zone is at a different site from the antibody capture zone, the arrangement of the device is generally such that antibodies in the sample migrate to the antibody capture zone. The required reagents are then applied in the appropriate order at designated application zones, which may or may not be the same as the sample application zone. Again, if the or any reagent application zone is at a different site from the antibody capture zone, the arrangement of a device is generally such that the reagent(s) migrate to the antibody capture zone, where any antigen-antibody complex formed is detected. All or some of the reagents required for an immunoassay may be incorporated within a device, in liquid or dry form. If so, a device is generally arranged such that interactions between different parts of the device, which interactions may occur automatically during the operation of the device or may be brought about by the user of the device, bring the various

reagents into contact with one another in the correct sequence for the immunoassay to be carried out.

A wide variety of assay devices are described in the literature of immunoassays. Examples of membrane devices are described in U.S. Patents Nos. 4,623,461 and 4,693,984. Depending on their design and their speed of action, some assay devices are called "dipsticks" and some are called "rapid assay" devices. A "rapid assay" device generally provides a result within ten minutes of the application of sample. (A typical microtitre plate or bead assay requires incubation steps, and generally takes at least an hour to provide a result.) Accordingly, although assay devices are generally more expensive than microtitre or bead format assays, they have particular uses in clinical testing, for example, when a result is required rapidly, for example, in the case of emergency surgery.

Assay devices have the particular advantage that they can be used without the need for sophisticated laboratory facilities or even without the need for any laboratory facilities. They may therefore be used for "on the spot" testing, for example, in an emergency room, in a doctor's surgery, in a pharmacy or, in certain cases, for home testing. They are particularly useful in territories where laboratory facilities are few and far between.

Factor XII and its activated form, Factor XIIa, are involved in blood coagulation and other contact systems, also known as contact phase systems, for example, fibrinolysis, complement cascade, inflammation and vasodilation, see Jacobsen S. and Kriz M., Br J Pharmacol., 29, 25-36, 1967; Kurachi K et al, Biochemistry, 19, 1330-

8 1980; Radcliffe R et al, Blood, 50, 611-7, 1977; Ghebrehiwet B et al, J Clin Invest,71, 1450-6. 1983; Z Toossi et al, Proc Natl Acad Sci USA, , 89, 11969-72, 1992; Wachtfogel YT et al, Blood 67, 1731-7, 1986; Wachtfogel YT et al, Thromb Haemost, 80, 686-91, 1998; and Schreiber et al AD, J Clin Invest. ,52, 1402-9, 1973.

As Factor XII and its activated form, Factor XIIa are involved in haemocoagulation and have a role in maintaining vascular wholeness and blood pressure, in influencing various functions of endothelial cells, in control of fibrinolysis and in maintaining the constitutive anticoagulant character of the intravascular space, measurement of cellular Factor XIIa is useful in investigations of those systems, including for example, fibrinolysis, complement cascade, inflammation and vasodilation. Clinical and experimental studies indicate that the contact system, which includes Factor XIIa, is involved in acute and chronic inflammation, shock of different aetiologies, diabetes, allergy, thrombo-haemorrhagic disorders including disseminated intravascular blood coagulation, oncological diseases, cardiovascular conditions, for example, myocardial infarction, angina and acute coronary syndrome angiogenesis, sepsis, spontaneous abortion and thromboembolism.

Determination of cellular Factor XII and fragments thereof, including Factor XIIa, are therefore useful in clinical and scientific investigations of such conditions, including diagnosing, predicting susceptibility to, monitoring and monitoring treatment of disorders in which the contact system is involved, including acute and chronic inflammation, shock of

different aetiologies, diabetes, allergy, thrombo-haemorrhagic disorders including disseminated intravascular blood coagulation and thromboembolism, oncological diseases, cardiovascular conditions, for example, myocardial infarction, angina and acute coronary syndrome, angiogenesis, sepsis, and spontaneous abortion.

Detection or determination of cellular Factor XII or a fragment thereof, for example, Factor XIIa, is therefore useful as an aid to diagnosing or monitoring diseases and disorders in which the amount of cellular Factor XIIa is different from that in healthy subjects. Changes in the level of cellular Factor XIIa may be indicative of any of the conditions mentioned above. Changes in level in a subject with time may be indicative of change in the condition, for example, exacerbation of the condition, or improvement, for example, in response to therapy. Such methods of diagnosis and monitoring are part of the present invention.

We have made the surprising observation that an individual who was considered to be "totally Factor XII deficient" as determined by a conventional immunoassay for Factor XII and by the conventional clotting assay (Griffith et al. loc. cit). was found to have cellular Factor XIIa, see Example 5 and Figures 7 to 10. Circulating Factor XII originates from the liver. As the "Factor XII deficient individual has no circulating Factor XII in the aqueous phase, the cellular Factor XIIa could not have been formed by adsorption of aqueous phase Factor XII or XIIa, therefore the cellular Factor XII and XIIa must have been produced by another source. It is considered that it is likely is that there is production of Factor XII in other cell lines, for example,

lymphocytes or megakaryocytes. This is not only of scientific interest, it has clinical implications.....

The present invention provides a monoclonal antibody that is capable of binding to cellular Factor XII or a fragment thereof, for example, Factor XIIa, for example, a monoclonal antibody having the same or similar properties as mAb 2/215 in relation to binding to Factor XIIa, especially to cellular Factor XIIa, but not including mAb 2/215 itself.

A monoclonal antibody that is capable of binding to cellular XII or a fragment thereof, for example, Factor Factor XIIa, for example, a monoclonal antibody having the same or similar properties as mAb 2/215 in relation to binding to Factor XIIa, especially cellular Factor XIIa, may be produced by methods that are known per se. Resulting antibodies are screened for those having the desired characteristics.

It may be useful to use monoclonal antibody 2/215 as a reference antibody in the screens for antibodies that bind to cellular Factor XIIa. A selected antibody may have binding characteristics for cellular Factor XIIa that are the same as or similar to those of mAb 2/215.

The antigen used to raise the antibodies is Factor XII or a fragment thereof. An antigenic fragment of Factor XII may itself be immunogenic or may be too small to be immunogenic, in which case it may be converted into an immunogen, for example, by conjugation to another peptide, for example, as described below. The term "an antigenic fragment of Factor XII" as used herein includes both a fragment, for example, a peptide, and an

immunogenic form of such a fragment if it is not itself immunogenic.

An antigenic fragment of Factor XII may be Factor XIIa, for example, Factor α-XII or Factor β-XIIa or a fragment thereof, for example, a peptide that is a fragment of Factor βXIIa that is or that includes at least one antigenic determinant capable of recognising anti-Factor βXIIa.

Methods of preparing immunogens are known to those in the art. Any of these methods may be utilised to render immunogenic or to improve the immunogenicity of Factor XII or antigenic fragment thereof, see also WO90/08835.

For example, Factor βXIIa may be used as the immunogen to raise anti-Factor XIIa monoclonal or polyclonal antibodies. Factor βXIIa may be produced by a method which comprises first isolating Factor XII from fresh or freshly frozen plasma, for example, using a combination of ammonium sulphate precipitation and anion exchange chromatography for example, according to the method described by K. Fujikawa and E. W. Davie (Methods in Enzymol, 1981, 80, 198-211). Methods for converting Factor XII to Factor βXIIa and isolating Factor βXIIa from the resulting mixture are described by K. Fujikawa and B. A. McMullen (Journal of Biol.Chem., 1983, 258, 10924-10933) and B. A. McMullen and K. Fujikawa (Journal of Biol. Chem. 1985, 260, 5328). To obtain Factor βXIIa, Factor XII is generally subjected to limited cleavage, for example, by chemical or enzymatic digestion, for example, using trypsin or a trypsin-like enzyme, generally in a highly diluted form, for example, in a

molar ratio of trypsin:Factor XII of 1:500, for example, in a weight ratio trypsin:Factor XII of 1:75 and the cleavage products separated, generally by chromatography.

An antigenic fragment of Factor βXIIa may be produced by degradation of Factor βXIIa by enzymatic or chemical means. For example the disulphide-linked light chain peptide of Factor βXIIa can be obtained by reduction and carboxymethylation of Factor βXIIa and isolation of the fragment by chromatography (K. Fujikawa and B. A. McMullen Journal of Biol. Chem. 1983, 258, 10924). Alternatively, an antigenic fragment of Factor βXIIa may be produced if its amino acid sequence is known, synthetically, as may Factor βXIIa itself. Any of the many known chemical methods of peptide synthesis may be used, especially those utilising automated apparatus.

An antigenic fragment of Factor βXIIa may be produced using the techniques of recombinant DNA technology, as may Factor βXIIa itself. Cool et al, 1985 and 1987, loc. cit. have characterised a human blood coagulation Factor XII cDNA and gene. Recombinant production may be achieved by known methods, see for example, WO90/08835.

Unless specified otherwise, the terms "Factor βXIIa" and "βXIIa" as used herein include antigenic fragments of the Factor βXIIa molecule.

A monoclonal antibody for use according to the present invention must be capable of binding cellular Factor XII or a fragment thereof, for example, Factor XIIa. For example, it may be capable of binding to cellular Factor αXIIa, that is to say, it may be capable of recognising an antigenic determinant characteristic of αXIIa, or it

may be capable of binding to cellular fragments of αXIIa, for example, ßXIIa. An immunoassay using an appropriate antigen may be used to determine the specificity of the antibody.

If desired, a monoclonal antibody for use according to the present invention may bind to both cellular Factor XII and cellular Factor XIIa, or may bind to cellular Factor XIIa but show no significant binding to cellular Factor XII. In the latter case, the corrected cross-reactivity with Factor XII is, for example, 0.1% or less. A factor to take into consideration in assessing the cross-reactivity of an antibody of the invention with Factor XII is that even "pure" Factor XII preparations are almost inevitably contaminated with small amounts of Factor XIIa (Silverberg and Kaplan, Blood 60, 1982, 64-70). WO90/08835 gives details of methods of assessing the corrected cross-reactivity with Factor XII. Unless specified otherwise, the term "cross reactivity" is used herein to mean the corrected cross reactivity.

Methods used to produce monoclonal antibodies are well known, see for example, Methods in Enzymology, H. Van Vunakis and J. J. Longone (Eds) 1981, 72(B) and ibid, 1983 92(E).

Monoclonal antibodies may be produced, for example, by a modification of the method of Kohler and Milstein (G. Kohler and C. Milstein, Nature, 1975, 256, 495): female Balb/C or C57/BI0 mice are immunised by intraperitoneal injection of Factor XII or an antigenic fragment thereof, for example, from 10 to 30 μg, generally 20 μg of Factor βXIIa or a corresponding amount of the other antigen. The Factor βXIIa or other antigen

is preferably conjugated to another protein molecule, for example, to a purified protein derivative of tuberculin or, preferably, to bovine thyroglobulin. The conjugation may be carried out, for example, by a carbodiimide method or by using a hetero-bifunctional reagent. The immunogen is generally presented in an adjuvant, preferably complete Freunds adjuvant. This procedure is generally repeated at intervals, generally using the same immunogen in the same dose, for example, at 3 week intervals the mice are boosted with 20 μg of conjugated Factor βXIIa in complete Freunds adjuvant until suitable response levels are observed. A pre-fusion boost is preferably given prior to sacrifice, for example, intravenously 3 days prior to sacrifice.

The antibody response is monitored, for example, by RIA antisera curve analysis using, for example, ^{125}I radiolabelled Factor XII or a fragment thereof, for example, radiolabelled Factor βXIIa or another Factor βXIIa antigen prepared by the chloramine-T method (P. J. McConahey and F. J. Dixon, Int. Arch. Allergy Appl. Immunol, 1966, 29, 185). Purity is confirmed, for example, by using autoradiography, for example, of SDS-PAGE gels run under reducing conditions.

Immune mouse spleen cells are then fused with myeloma cells, for example, NSO mouse myeloma cells, for example in the presence of 40-50% PEG 4,000 or 50% PEG 1500. The cells are then seeded in wells of culture plates and grown on a selective medium. The supernatants are tested for reactivity against the corresponding purified Factor XII antigen, for example, in the case of a Factor βXIIa antigen, purified Factor βXIIa or other βXIIa antigen, for example, by a solid phase enzyme immunoassay, for

example, using peroxidase-labelled anti-mouse IgG. All wells showing specificity for the antigen used for testing are generally taken for further secondary screening. The secondary screening consists, for example, of screening all specific antibodies for binding in solution to the appropriate antigen, for example, in the case of a Factor βXIIa antigen, Factor βXIIa or a Factor βXIIa antigenic fragment that has been radiolabelled. These are preferably titrated to determine the antibody dilution required for 50% B max. Dose-response curves against cold, that is to say non-labelled antigen are generated, and are preferably also generated against Factor XII (if no cross-reactivity with Factor XII is desired), plasmin and fibronectin. The extent of cross reaction may be determined according to the following formula:

$$\frac{\text{Weight of Cold Standard Antigen to Achieve 50\% B max}}{\text{Weight of Cross-Reactant to achieve 50\% B max}} \times 100$$

Those antibodies showing an appropriate level of binding to the desired antigen, Factor βXIIa, for example, having affinity constants of at least $10^{10}M^{-1}$ are generally taken forward for cloning.

Successful clones are generally isotyped. The cells are then preferably sub-cloned by limiting dilution and again screened, generally using an enzyme immunoassay, for the production of antibodies to the desired antigen, for example Factor βXIIa. A selected sub-clone from each cloning may also be evaluated with respect to specificity and dose response using a radioimmunoassay or ELISA.

The next screening step is to determine the ability of the antibody produced by a selected clone to bind to cellular Factor XII or a fragment thereof, for example, Factor XIIa. This may be done by flow cytometry, for example, using mAb 2/215 for comparison.

If it is desired that the antibody should show no significant binding to Factor XII, an appropriate screening procedure is carried out. The antibodies may be screened for those showing a pre-determined apparent cross reactivity to Factor XII, preferably of 1.5% or less, for example 1% or less, for example 0.5% or less, for example, 0.1% or less.

Screening against non-cellular Factor XII or Factor XIIa is generally carried out first, but the two or optionally three screens may be carried out in any order.

Scatchard analysis may be done on the dose-response data to produce values for the affinity constants for each antibody.

Sub-cloned or cloned hybridoma cells may be injected intra-peritoneally into Balb/C mice for the production of ascitic fluid. The immunoglobulin may be precipitated from ascitic fluid, for example, at 4°C using saturated ammonium sulphate solution (equal volume). The precipitate is preferably purified, for example, it may be centrifuged, dissolved, for example, in 50mM Tris-HCl buffer pH 7.5 (volume equal to original ascites volume) and then dialysed against the same buffer. The immunoglobulin fraction may then be further purified by anion exchange chromatography, for example, the protein solution may be applied to a Mono-Q anion exchange column

(Pharmacia) and eluted using a salt gradient in the same buffer according to the manufacturer's recommendations. The fractions containing immunoglobulin are generally pooled and frozen at -20°C for storage. Alternatively, hybridoma cells may be grown in culture for antibody production and the antibody isolated essentially as described above for ascites fluid.

Although the hybridomas described herein are derived from mouse spleen cells, the invention is not limited to hybridomas of murine or part-murine origin. Both fusion partners (spleen cells and myelomas) may be obtained from any suitable animal. Recombinant antibodies may be produced. Antibodies may be brought into chimeric or humanized form, if desired. The hybridomas are preferably cultured in vitro.

The present invention also provides polyclonal antibodies, also called a polyclonal antiserum, that are capable of reacting with cellular Factor XII or a fragment thereof, for example, Factor XIIa. Such antibodies may be labeled and used for detection of captured cellular Factor XII or a fragment thereof, for example, Factor XII, in an ELISA.

The invention also provides a method for the production of such a polyclonal antiserum, which comprises administering Factor XII or a fragment thereof, for example, Factor XIIa, especially Factor βXIIa to an animal, obtaining serum from the animal, screening the serum for binding to cellular Factor XII or a fragment thereof, for example, Factor XIIa.

The following non-limiting Examples illustrate the present invention.

EXAMPLES

Microtitre plate assay

100μl aliquots of sample were added to wells of a microplate precoated with 2/215 Monoclonal antibody. After incubation for 60 minutes, the plates were washed with a borate buffered saline wash solution (pH 7.4). 100 μl of the relevant conjugate (alkaline phosphatase labelled antibody) was added to each well, and the plate was incubated for a further 60 minutes. After washing the plate again, 100 μl of phenolphthalein phosphate substrate was added. After a suitable incubation period, an alkaline Stop solution was added to inhibit further substrate conversion, and the absorbance was recorded at 550nm.

IMx assay

The Abbott IMx system is an automated immunoassay analyser designed to run assays using enzyme immunoassay and fluoresence polarisation immunoassay technologies.

The technique used in these Examples is microparticle enzyme immunoassay (MEIA). MEIA technology uses microparticles coated with a capture molecule (in this case an antibody) specific for the molecule being measured. The effective surface area of the microparticles and diffusion distance between analyte and solid phase result in improved assay kinetics, permitting MEIA assays to be completed more rapidly than many other immunoassays. The microparticles along with the bound analyte are separated from the reaction mixture by

binding irreversibly to the glass fibre matrix used in the MEIA reaction cell.

The reactants necessary for MEIA assays are

- Microparticles coated with a capture molecule (in this case monoclonal antibody 2/215)
- Alkaline phosphatase-labeled conjugate (in this case antibodies against activated Factor XII, either polyclonal antibodies or mAb 2/215)
- Fluorogenic substrate, 4-methylumbelliferyl phosphate (MUP)
- Reaction cell that contains a glass fibre matrix to which immune complex binds.

Other reagents such as a diluent and/or wash solution are also required.

The following is a description of the MEIA Reaction process.

1. The IMx system transfers sample and microparticles (coated with capture molecules) to the incubation well of the reaction cell. During an incubation period, analytes bind to the microparticles, creating an immune complex.
2. The IMx System transfers an aliquot of the immune complex to the inert glass fibre matrix of the reaction cell. The immune complex binds irreversibly to the glass fibre matrix. The IMx washes the matrix to remove unbound materials, and the immune complex is retained by the glass fibres whilst the excess reaction mixture flows rapidly through the large pores in the matrix.
3. The IMx system adds alkaline phosphatase labelled conjugate to the matrix. The conjugate binds to the

immune complex to complete the antibody-analyte-conjugate "sandwich". The IMx washes the matrix again.

4. The IMx system adds the flurogenic substrate 4-methylumbelliferyl phosphate (MUP) to the matrix. The conjugate catalyses the hydrolysis of 4-methylumbelliferyl phosphate (MUP) to 4-methylumbelliferone (MU)
5. The MEIA optics within the IMx instrument measure the rate at which the fluorescent product (MU) is generated on the glass fibre matrix. The rate at which MU is generated on the matrix is proportional to the concentration of the analyte in the test sample.

The protocol used for experiments described below is set out in Figure 1 of the accompanying drawings.

Example 1.

Blood was collected from a volunteer into two citrate tubes were and the red blood cells were separated by centrifugation at 1000g for 10 minutes. The plasma from these tubes was pooled to eliminate any collection tube variation. A proportion of the plasma was aliquotted as 1ml aliquots in Eppendorf tubes) and labeled "cell rich plasma".

The remainder of the plasma was aliquotted and then centrifuged at high speed (16000g for 10 minutes) in a microcentrifuge. The supernatant was separated and labeled "cell poor plasma". The precipitate was then washed by resuspending it in 100 mM Phosphate buffered saline, pH 7.4 (PBS) and centrifuging at 16000g for 10 minutes, following which the supernatant was discarded. The pelleted material was washed in the same manner a

further 2 times, following which it was resuspended in PBS and labeled "washed cells".

The three samples (cell rich plasma, cell poor plasma and washed cells) were then assayed using the microtitre plate assay described above, in which mAb 2/215 was used to capture cellular Factor XIIa and either labeled mAb 2/215 (2/215 conjugate) or labeled polyclonal antibody (polyclonal conjugate) was used for detection of captured cellular Factor XIIa.

The results obtained are shown in Table 1. The results with the 2/215 conjugate are expressed as absorbances as no standards were available for the dual 2/215 (capture antibody and conjugate antibody) assay. Plots of this data that has been normalized is shown in Figure 2.

Table 1. Results obtained for different sample types from microtitre plate XIIa assays with polyclonal and 2/215 conjugates.

Sample	XIIa MTP polyclonal conj	A550 MTP 2/215 conj
Cell poor plasma	3.5	0.209
Washed cells	<0.1	1.442
Cell Rich plasma	3.6	0.753

With the polyclonal conjugate significant responses are obtained for both the cell rich and cell poor plasmas,

but only a minimal response is obtained with the washed cells. In contrast when using the 2/215 conjugate, the maximal response is obtained with the washed cells, whilst the lowest response is obtained for the cell poor plasma.

Example 2.

Blood was collected from a volunteer into 6 citrate tubes and red blood cells were separated by centrifugation at 1000g for 10 minutes. The plasma from all tubes was pooled to eliminate any collection tube variation. A proportion of the plasma was aliquotted as 1ml aliquots in Eppendorf tubes and labeled "cell Rich Plasma".

The remainder of the plasma was aliquotted and then centrifuged at high speed (16000g for 10 minutes) in a microcentrifuge. The supernatant was separated and labeled "cell poor plasma". The precipitate was then washed by resuspending it in 100 mM Phosphate buffered saline, pH 7.4 (PBS) and centrifuging at 16000 G for 10 minutes, following which the supernatant was discarded. The pelleted material was washed in the same manner a further 2 times, following which it was resuspended in PBS and labeled "cell suspension".

The three samples (cell rich plasma, cell poor plasma and cell suspension) were then assayed using the microtitre plate assay as described in Example 1, using both the 2/215 conjugate and the polyclonal conjugate. The samples were also assayed using an assay for activated Factor XII using the Abbott IMx automated immunoassay instrument. The conjugates used in this case for detection of cellular Factor XIIa were alkaline phosphatase labeled mAbs 201/9 and 2/215, cf Example 1. Plots of data

obtained in the microtitre plate assay are shown in Figure 3, and plots of data obtained in the IMx assay are shown in Figure 4.

In the microtitre plate assay using the polyclonal conjugate, significant responses are obtained for both cell rich and cell poor plasma whilst a minimal response is obtained with the cell suspension, whereas when using the 2/215 conjugate the maximum response was obtained for the cell suspension. Much lower responses were obtained for cell rich and cell poor plasma when using the 2/215 conjugate, with cell poor plasma givin the lowest response.

In the IMx assay using the 201/9 conjugate, significant responses are obtained for both cell rich and cell poor plasma whilst a minimal response is obtained with the cell suspension. When the 2/215 conjugate is used in the IMx assay significant responses are seen with the cell suspension and cell rich plasma, with a much reduced response for cell poor plasma

Example 3. Flow cytometric analysis of cellular XIIa.
As an alternative means of assessing whether mAb 2/215 was binding to XIIa on cells within plasma, flow cytometry was employed.

mAb 2/215 was labeled with fluorescein isothiocyanate (FITC). This FITC labeled 2/215 antibody was incubated with cell rich plasma, and along with a control (no labeled antibody added) tested using flow cytometry. The resulting output is shown in Figure 5.

In Figure 5, the shift of the peak to the right upon the addition of FITC labeled antibody is indicative that the antibody is binding to cells in the plasma sample.

Example 4. Measurement of cellular XIIa by incubation with radiolabelled antibody.

A further method of demonstrating and quantitating (determining) cellular XIIa is by the addition of radiolabelled 2/215 antibody to whole blood or plasma samples, separating the cells by centrifugation and measuring the amount of radioactivity bound to this fraction.

Monoclonal antibody 2/215 was labeled with Iodine 125. Blood samples were obtained from 8 volunteers incubated with radiolabeled antibody. Red blood cells were removed by centrifugation at low centrifugal force (1000g), and other cells (including platelets and white blood cells) were separated by centrifugation at higher centrifugal force (16000g). The pelleted cells were washed by resuspending in 100 mM Phosphate buffered saline, pH 7.4 (PBS) and centrifuging at 16000g for 10 minutes, following which the supernatant was discarded. The pelleted material was washed in the same manner a further 2 times, following which it was resuspended in PBS and labeled "cell suspension".

Radioactivity associated with this cellular material, and of total amount of antibody bound to XIIa (as opposed to free antibody) was measured, and the proportion of XIIa in the cellular fraction was calculated accordingly.

In Table 2, the percentage of added antibody bound to cellular XIIa and the proportion of the antibody bound to

XIIa associated with the cellular fraction is shown. It can be seen that there is a significant but variable amount of cellular XIIa. In Figure 6 is a graphical representation of the relative cellular XIIa concentration for 8 individuals

Table 2. Percentage of added radiolabeled antibody bound to cellular XIIa and the proportion of the antibody bound to XIIa associated with the cellular fraction

Donor	% of added antibody bound to cellular XIIa	Proportion (%) of total bound antibody in cellular fraction
5	2.99	23
6	3.96	32
7	2.30	28
8	3.77	34
9	1.81	20
10	0.68	11
11	0.86	12
12	1.20	14

Example 5. Cellular XIIa in Factor XII deficient individuals.

Citrated blood was collected from a "normal" volunteer and from an individual considered totally deficient in Factor XII (demonstrated by a Factor XII antigen ELISA using antibodies available from ERL, 15 Skelty Rd, Swansea, UK) and by measurement of Factor XII using a clotting assay (Griffin, J. H. & Cochrane, C. G., in Methods in Enzymology, Academic Press (New York) 45, 56-65, 1976). Cells were separated by centrifugation at 1000g for 10 minutes. The plasma from all tubes was

pooled for each individual to eliminate any collection tube variation. A proportion of the plasma was aliquotted as 1ml aliquots in Eppendorf tubes and labeled "cell rich plasma".

The remainder of the plasma was aliquotted and then centrifuged at high speed (16000g for 10 minutes) in a microcentrifuge. The supernatant was separated and labeled "cell poor plasma". The precipitate was then washed by resuspending it in 100 mM Phosphate buffered saline, pH 7.4 (PBS) and centrifuging at 16000 G for 10 minutes, following which the supernatant was discarded. The pelleted material was washed in the same manner a further 2 times, following which it was resuspended in PBS and labeled "cell suspension".

The samples (cell rich plasma, cell poor plasma and cell suspension) were then assayed using the microtitre plate assay with the 2/215 conjugate and the polyclonal conjugate as described in Example 1. The samples were also assayed using the assay for activated Factor XII using the Abbott IMx automated immunoassay instrument as described in Example 2. The conjugates used in this case for detection of cellular Factor XIIa were peroxidase labeled mAbs 201/9 and 2/215, cf Example 1.

The surprising observation was made that there was a cellular Factor XIIa response from the individual who was "totally Factor XII deficient" (examples of data in Figures 7 to 10). Circulating Factor XII originates from the liver. As the "Factor XII deficient individual has no circulating Factor XII in the aqueous phase, the cell -bound Factor XIIa could not have been formed by adsorption of aqueous phase Factor XIIor XIIa, therefore

the cellular Factor XII and XIIa must have been produced by another source. It is considered that it is likely is that there is production of Factor XII in other cell lines, for example, lymphocytes or megakaryocytes.

CLAIMS:

1. A method which comprises detecting or determining Factor XII or a fragment thereof in a sample comprising cells obtained from a mammalian subject.

2. A method as claimed in claim 1, wherein the fragment of Factor XII is Factor XIIa.

3. A method as claimed in claim 2, wherein a chromogenic assay is used to detect or determine Factor XIIa thereof.

4. A method as claimed in claim 1 or claim 2, wherein an immunoassay is used to detect or determine Factor XII or a fragment thereof.

5. A method as claimed in claim 4, wherein the cells are contacted with a labelled antibody that is capable of binding to cellular Factor XII or a fragment thereof and any resulting an antigen-antibody complex is detected or determined.

6. A method as claimed in claim 5, wherein the antibody is mAb 2/215, which is produced by hybridoma 2/215, deposited at the European Collection of Animal Cell Cultures, Divisional of Biologics, PHLS Centre for Applied Microbiology and Research, Porton Down, Salisbury SP4 0JG, England (known as ECACC) under the deposit number 90011606, or is another monoclonal antibody having the same or similar XIIa binding properties for cellular Factor XIIa as mAb 2/215.

7. A method as claimed in any one of clams 4 to 6, wherein the antibody is labelled with a fluorescent label and the detection or determination is carried out by flow cytometry.

8. A method as claimed in any one of claims 1 to 7, wherein the sample is a sample of a body fluid.

9. A method as claimed in claim 8, wherein the body fluid is whole blood or plasma.

10. A method as claimed in any one of claims 1 to 7, wherein the cells are isolated cells.

11. A method as claimed in claim 10, wherein the cells are isolated from blood.

12. A method as claimed in claim 10 or claim 11, wherein the cells are isolated by centrifugation and washing.

13. A method of diagnosing or monitoring a disease or disorder in a subject, in which disease or disorder the amount of cellular Factor XII or a fragment thereof is different from that in a subject not having the disease or disorder, which comprises determining Factor XII or a fragment thereof in a sample comprising cells obtained from the subject under investigation.

14. A method as claimed in claim 13, which comprises comparing the level of Factor XII or a fragment thereof determined with levels of Factor XII or a fragment thereof determined in a sample of cells obtained from a subject not having the disease or disorder.

15. A method as claimed in claim 13 or claim 14, wherein the disease or disorder is a disease or disorder of the coagulation system.

16. A method as claimed in claim 13 or claim 14, wherein the disease or disorder is associated with inflammation or the inflammatory response.

17. A method as claimed in claim 13 or claim 14, wherein the disease or disorder is sepsis.

18. A method as claimed in claim 13 or claim 14, wherein the disease or disorder is acute or chronic inflammation, shock of different aetiologies, diabetes, allergy, a thrombo-haemorrhagic disorder, an oncological diseases, or a cardiovascular condition.

19. A method as claimed in claim 13 or claim 14, wherein the disease or disorder is myocardial infarction, angina, acute coronary syndrome, disseminated intravascular blood coagulation or thromboembolism

20. A method as claimed in claim 13 or claim 14, wherein the disease or disorder is spontaneous abortion.

21. A method as claimed in any one of claims 13 to 20, wherein cellular Factor XII is determined by a method as claimed in any one of claims 1 to 12.

22. A monoclonal antibody having the same or similar cellular Factor XIIa binding properties as mAb 2/215, other than mAb 2/215.

23. A method for producing a monoclonal antibody that binds to cellular Factor XII or a fragment thereof which comprises raising monoclonal antibodies against Factor XII or a fragment thereof and screening the antibodies against cellular Factor XII or a fragment thereof.

24. A method as claimed in claim 23, wherein Factor XIIa is used to raise the antibodies and cellular Factor XIIa is used for screening.

25. A method as claimed in claim 23 or claim 24, wherein mAb 2/215 is used as a reference antibody in screening.

ABSTRACT

FACTOR XII VARIANT

Detection or determination of cellular Factor XII or a fragment thereof, for example, cellular Factor XIIa is useful as an aid to diagnosing or monitoring diseases and disorders in which the amount of cellular Factor XIIa is different from that in healthy subjects. Changes in the level of cellular Factor XIIa may indicate, for example, changes in the coagulation system. Changes in level may be associates with inflammation or the inflammatory response.

60μl microparticles and 90μl sample added to reaction well

⇓

Reaction cell fibre matrix washed with 50μl prewash

⇓

Sample and microparticles incubated for 20 min.

⇓

100μl of 150μl sample/microparticle mix added to reaction cell fibre matrix

⇓

Reaction cell fibre matrix washed with 2 shots of 100μl MEIA buffer
(3 second delay between shots)

⇓

60μl of appropriate conjugate added to reaction cell fibre matrix

⇓

20min. incubation with conjugate on reaction cell fibre matrix

⇓

Reaction cell fibre matrix washed with 2 shots of 100μl MEIA buffer
(3 second delay between shots)

⇓

65μl MUP substrate added to Reaction cell fibre matrix

⇓

3 second delay then read

Figure 1

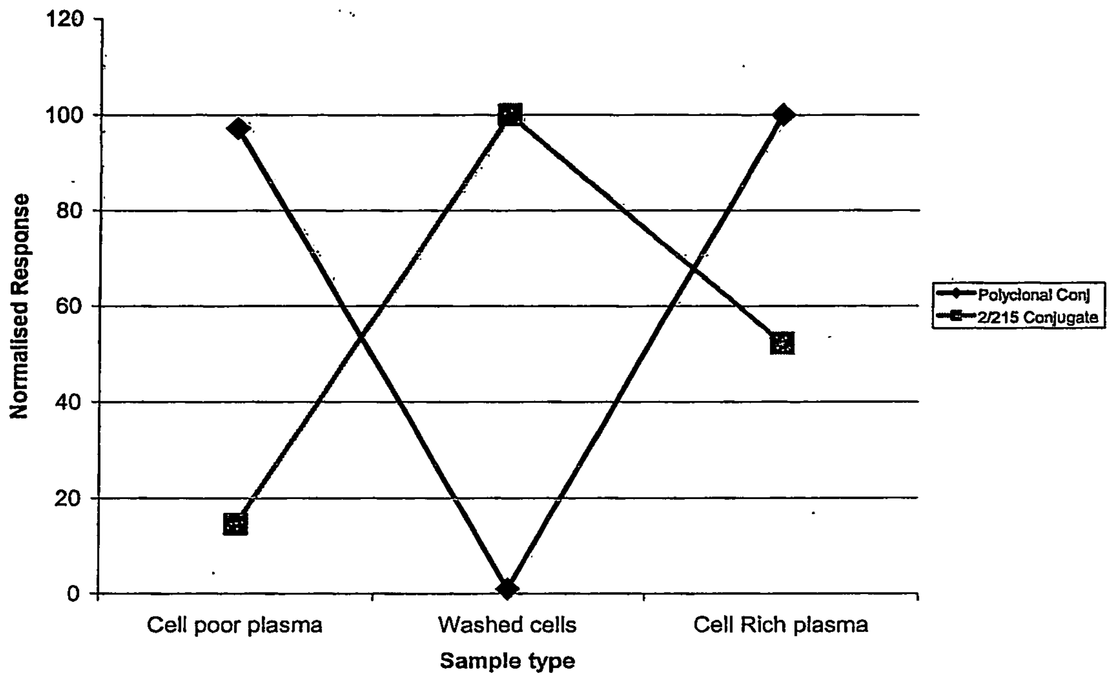

120
100
80
60
40
20
0
Normalised Response
Cell poor plasma
Washed cells
Cell Rich plasma
Sample type
Polyclonal Conj
2/215 Conjugate

Figure 2

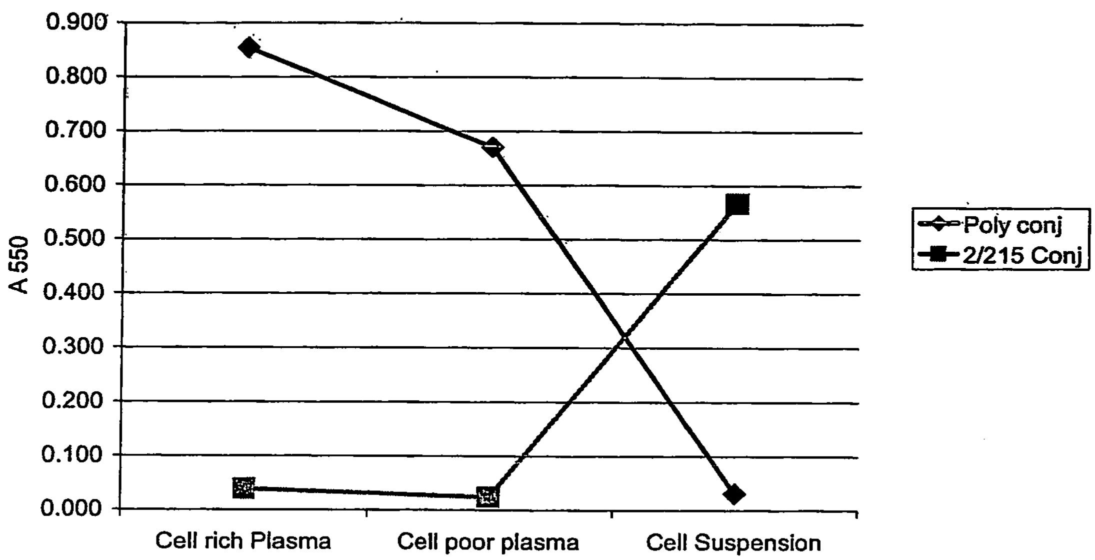

0.900
0.800
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
A 550
Cell rich Plasma
Cell poor plasma
Cell Suspension
Poly conj
2/215 Conj

Figure 3

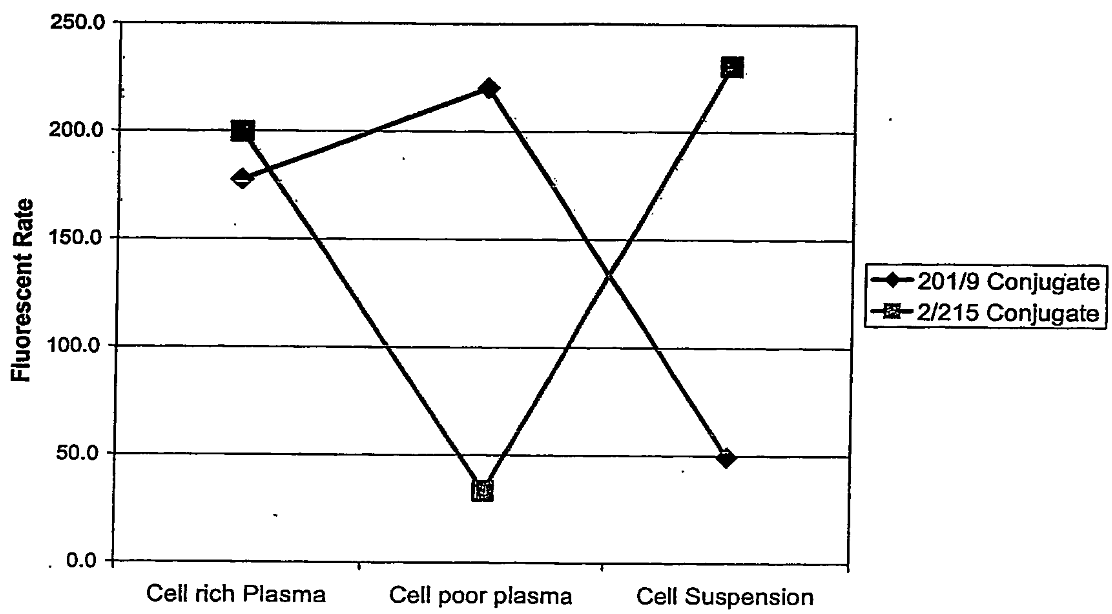
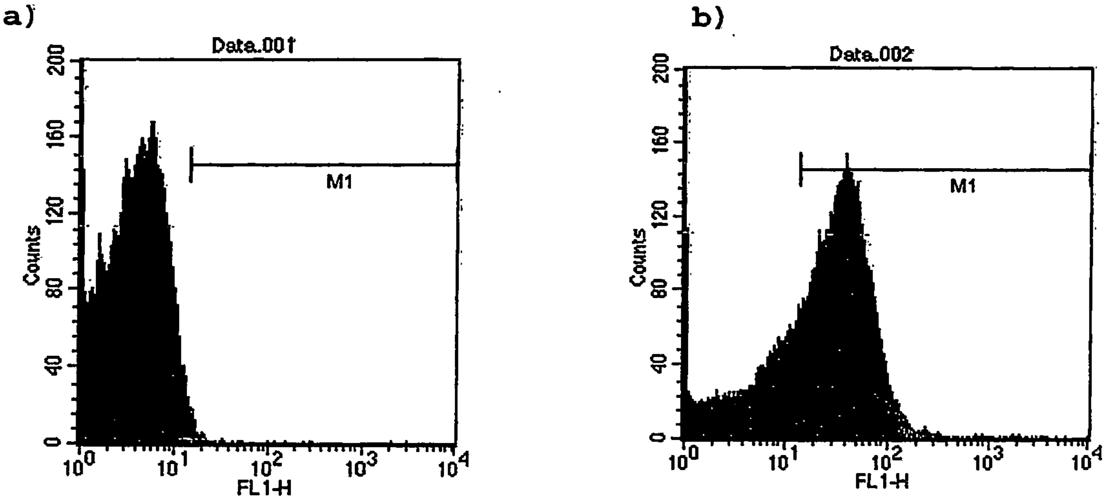

250.0
200.0
150.0
100.0
50.0
0.0
Fluorescent Rate
Cell rich Plasma
Cell poor plasma
Cell Suspension
201/9 Conjugate
2/215 Conjugate

Figure 4

a) b)



File: Data.001

Marker	% Total	Mean	Median
All	100.00	4.45	3.62
M1	0.58	49.90	15.96

File: Data.002

Marker	% Total	Mean	Median
All	100.00	33.56	24.80
M1	66.19	47.91	36.85

Figure 5

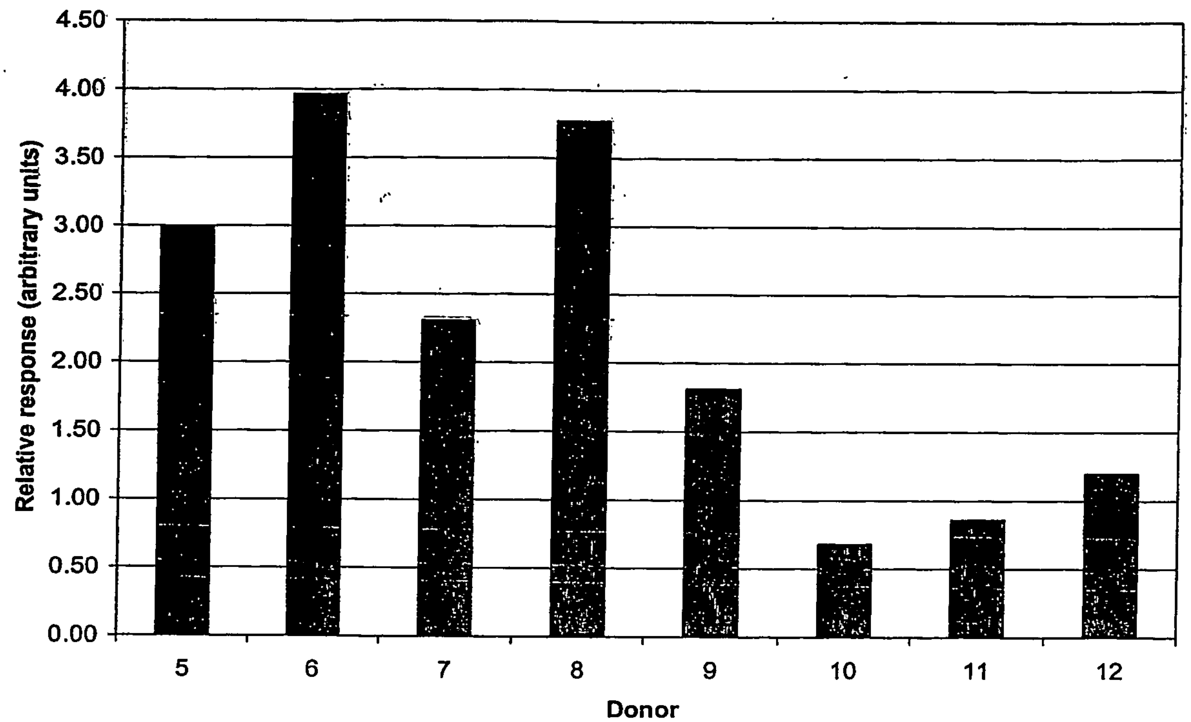

4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Relative response (arbitrary units)
5
6
7
8
9
10
11
12
Donor

Figure 6

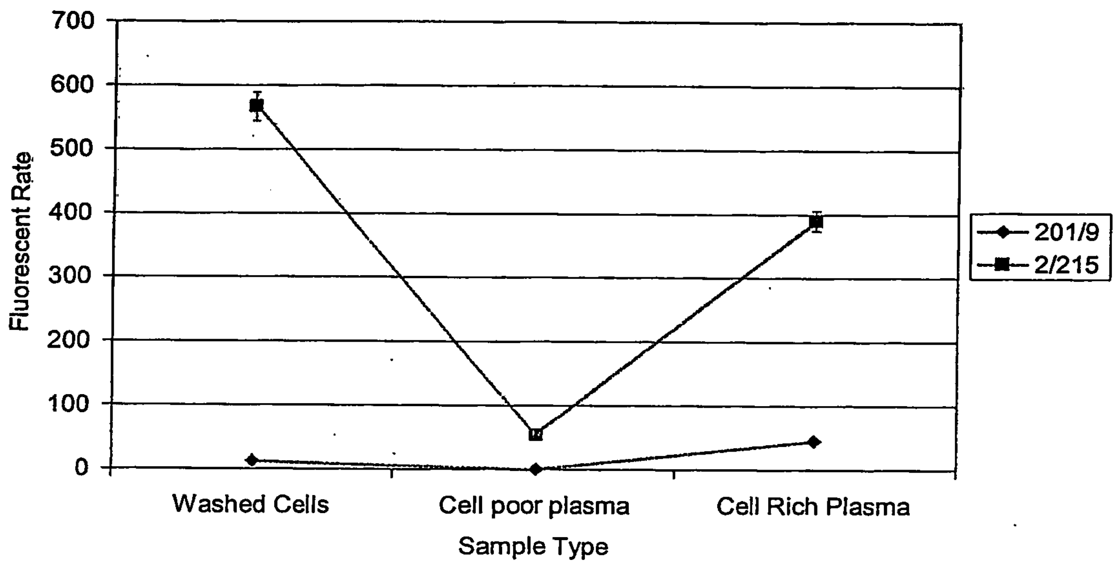

700
600
500
400
300
200
100
0
Fluorescent Rate
201/9
2/215
Washed Cells
Cell poor plasma
Cell Rich Plasma
Sample Type

Figure 7

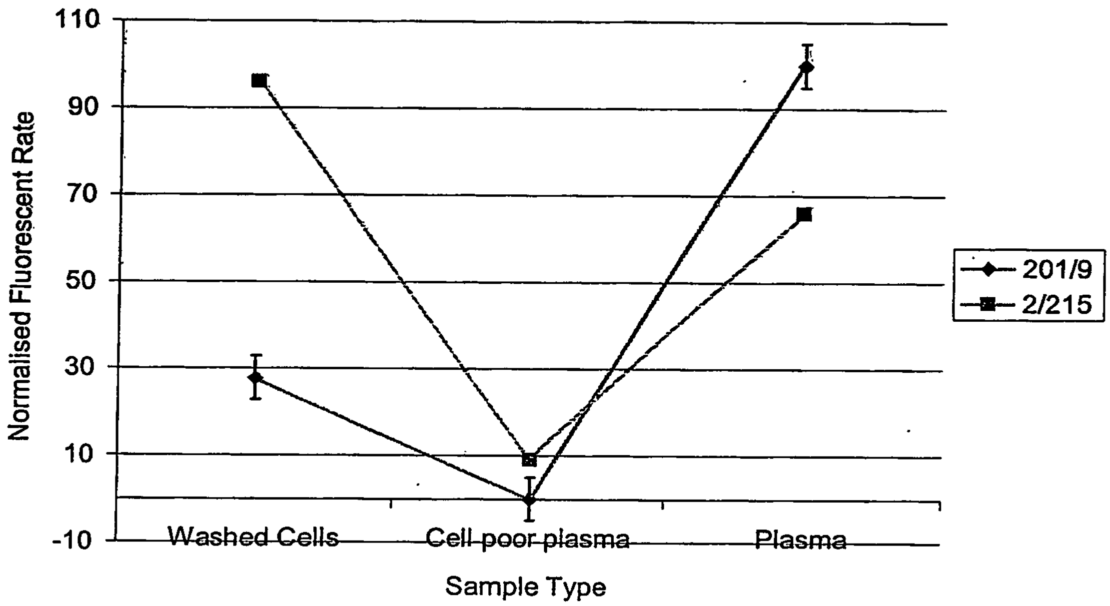

110
90
70
50
30
10
-10
Normalised Fluorescent Rate
Washed Cells
Cell poor plasma
Plasma
Sample Type
201/9
2/215

Figure 8

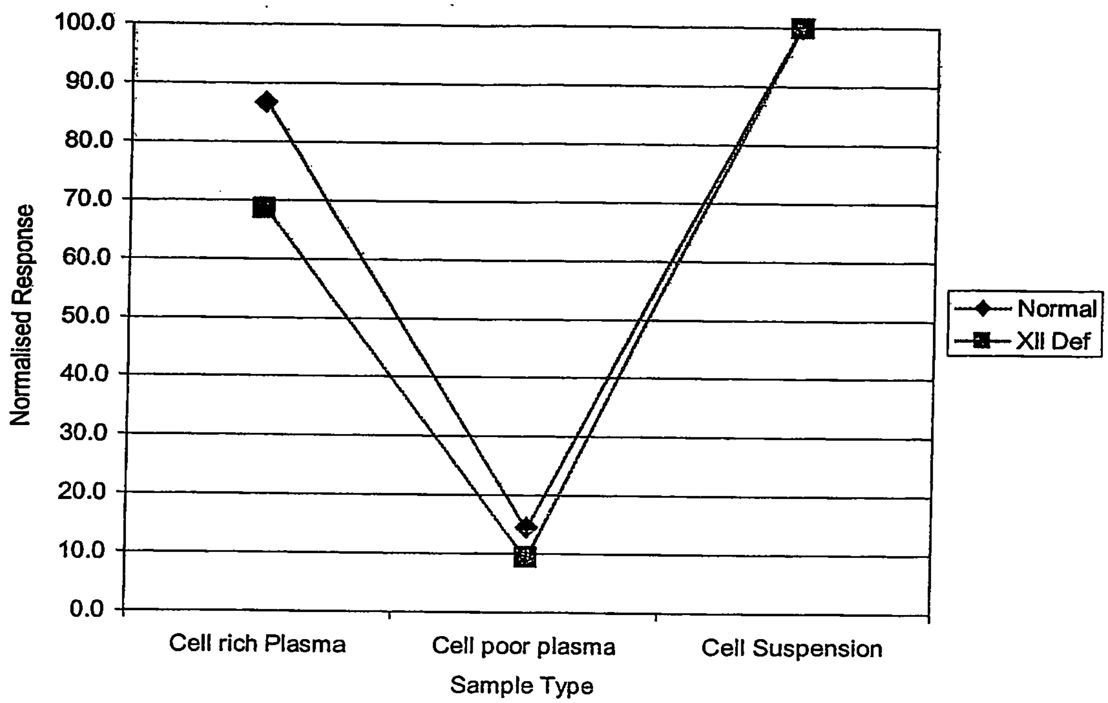
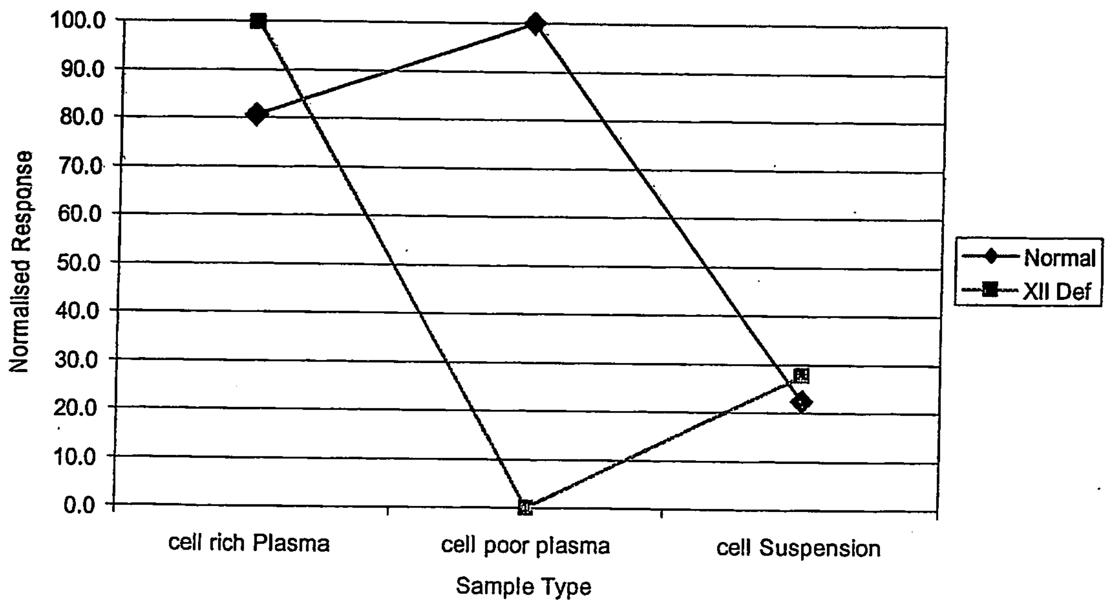

100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Normalised Response
Normal
XII Def
Cell rich Plasma
Cell poor plasma
Cell Suspension
Sample Type

Figure 9



100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Normalised Response
cell rich Plasma
cell poor plasma
cell Suspension
Sample Type
Normal
XII Def

Figure 10